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(57) Abstract

Binding compositions which selectively bind to a marker specifically found on Th2 cells and various methods of use of the binding compositions are provided. In particular, the method makes use of fact that the CCR8 chemokine receptor is selectively expressed on the Th2 cell subset. Preferably, the binding composition is a monoclonal antibody specific for CCR8.

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Th2 CELL DEPLETION; COMPOSITIONS; METHODS

The present filing claims priority from provisional U.S. patent application USSN 60/065,392, filed November 13, 1997.

Field of the Invention

The invention relates generally to methods of purifying or depleting Th2 cells and compositions used for such, and more particularly, to methods of treating diseases or conditions associated with elevated or depleted populations of Th2 cells.

BACKGROUND

The immune system consists of a wide range of distinct cell types, each with important roles to play. See Paul (ed. 1993) Fundamental Immunology, 3d ed, Raven Press, New York. The lymphocytes occupy central stage because they are the cells that determine the specificity of immunity, and it is their response that orchestrates the effector limbs of the immune system. Two broad classes of lymphocytes are recognized: the B lymphocytes, which are precursors of antibody secreting cells, and the T (thymus-dependent) lymphocytes. T lymphocytes express important regulatory functions, such as the ability to help or inhibit the development of specific types of immune response, including antibody production and increased microbicidal activity of macrophages. Other T lymphocytes are involved in direct effector functions, such as the lysis of virus infected-cells or certain neoplastic cells.

T cells may be subdivided into two distinct classes based on the cell-surface receptors they express. T cells, particularly the CD4+ T cells, are the major regulatory cells of the immune system. These CD4+ cells tend to differentiate into the Th2 or Th1 subsets. The Th2 cells

are effective in helping B cells develop into antibody producing cells, while the Th1 cells effectively induce cellular immune responses, e.g., cell mediated immunity, delayed type hypersensitivity, macrophage activation, and production of opsonizing antibodies. The Th1 cell subsets produce IL-2, IFN-γ, TNF-α, and lymphotoxin, but little or no IL-4, IL-5, or IL-10. In contrast, Th2 cell subsets produce IL-4, IL-5, IL-6, IL-10, and IL-13, but little or no IL-2 or IFN-γ. The different cytokine patterns lead to markedly different immune responses.

Another subset of the T cells, the Tr1, have also been described. See, e.g., Groux, et al. (1997) Nature 389:737-742 (1997); Groux, et al. (1997) J. Immunol. 158;5627-5631; Groux, et al. (1996) J. Exp. Med. 184;19-29.

While the different types of cells can be distinguished, practical positive selection markers have not yet been identified. The availability of methods and/or reagents useful to specifically identify, select, or eliminate one or the other subset will find use both diagnostically and therapeutically. The present invention provides these.

SUMMARY OF THE INVENTION

The present invention is based, in part, upon the surprising discovery that the CCR8 chemokine receptor is specifically expressed on the Th2 subsets of T cells, virtually to the exclusion of other cell types. Thus, reagents which specifically bind to the chemokine receptor, e.g., ligand or antibodies, will affect only these cell subsets.

In particular, the agonist signaling of Th2 cells can be achieved using CCR8 agonists. Conversely, antagonists of CCR8 should block Th2 signaling. So the reagents will be useful in specifically activating or blocking the Th2 subset. This provides means to specifically attract or block Th2 trafficking, apoptosis, etc., and to redirect a Th2 to Th1 response, or vice versa.

Thus, the invention provides a substantially pure CCR8+ population of cells which consists essentially of activated Th2 cells. In various embodiments, the population will produce low amounts of the cytokines IL-2 and IFN-γ, and produce high amounts of IL-4 and IL-10; will bind anti-CCR8 antibody or antiserum; or will be prepared by Fluorescent Activated Cell Sorting with a labeled CCR8 selective: ligand; antibody; or binding compound comprising the antigen binding portion from an antibody which selectively binds CCR8. Preferably, the ligand is I-309 or TCA3; or the antibody binds to human CCR8.

In other embodiment, the invention provides a method of selectively labeling activated Th2 cells comprising contacting a population containing those cells to a reagent which specifically binds to a CCR8. In certain embodiments, the population comprises Th1 cells; or the method further involves selectively depleting the activated Th2 cells from the population, e.g., by killing of those cells expressing CCR8; or by FACS sorting of those activated Th2 cells. The depletion may use a toxic conjugate with: a ligand specific for CCR8 binding; a binding compound comprising an antigen binding portion from an antibody which selectively binds to a mammalian CCR8; or a binding composition comprising antigen binding portions from antiserum which selectively binds to a mammalian CCR8. Preferably, the mammalian CCR8 is human CCR8 and the antibody is a monoclonal antibody. Cells made by the method are also embraced.

Another embodiment includes a method of modulating a physiological signal specifically to activated Th2 cells, comprising contacting the activated Th2 cells with a CCR8 agonist or antagonist. For example, the modulating may be blocking, e.g., by contacting with a CCR8 antagonist, e.g., an antibody. The antagonist antibody may bind to either a CCR8 ligand or to CCR8. Alternatively, the modulating may be inducing with an agonist, e.g., by contacting with a CCR8 signaling ligand, e.g., I-309 or TCA3. The modulating may be directing a response between a Th1 and Th2 response,

where the contacting is with a CCR8 antagonist or CCR8 signaling agonist. In preferred embodiments, the physiological signal is a proliferation, apoptotic, or differentiation signal. The contacting may be in combination with another chemokine or cytokine agonist or antagonist, including IL-12, an IL-12 antagonist, IL-1γ, or an IL-1γ antagonist.

DETAILED DESCRIPTION OF THE INVENTION

I. General

The invention is based, in part, on the surprising discovery that a chemokine receptor, CCR8, is expressed predominantly on Th2 cells. Its expression level on other cells is very low or even undetectable, especially on other T cell types.

The chemokines are a sub-family of chemoattractant cytokines that were classically characterized by their ability to mediate leukocyte trafficking by binding to specific G-protein linked seven transmembrane spanning receptors, or GPCRs. Chemokines are divided into four groups based on the primary sequence of the first two cysteines: the CXC, CC, C, and the newly discovered, CX3C families. The CXC and C families are effective predominantly on neutrophils and lymphocytes, respectively. The CC chemokines are preferentially effective on macrophages, lymphocytes, and eosinophils.

Only about half of the chemokines have been paired to respective receptors. Some seem to bind to more than one receptor. The matching of orphan receptors with the many chemokines is an ongoing process. The matching of the ligands with receptors often provide useful insight into the physiological functions of the individual chemokines, often because the distribution of the receptors is quite limited.

The human CCR8 receptor, which was an orphan GPCR known under the names Ter1 (Napolitano, et al. (1996) J.

Immunol. 157:2759-2763), ChemR1 (Samson, et al. (1996) Genomics 36:522-526), or CKR-L1 (Zabellos, et al. (1996) Biochem. Biophys. Res. Commun. 227:846-853), has been known for some time. See GenBank accession numbers AF005210 (partial) and U45983. The ligand for the CCR8 chemokine receptor has been identified, in humans, the I-309 protein (Roos, et al. (1997) J. Biol. Chem. 272:17251-17254; and Tiffany, et al. (1997) J. Exp. Med. 186:165-170; GenBank Accession Numbers M57502 and M57506), and in mouse, the TCA-3 (GenBank Accession Numbers M17957 and X52401). However, the distribution of the CCR8 had been characterized as lymphoid specific, and particularly to the thymus. Napolitano, et al. (1996) J. Immunol. 157:2759-2763. The CCR8 distribution in the peripheral lymphocytes has not yet been well characterized until the present studies leading to this invention.

II. Specific Binding Compositions

A. Antibodies

The present invention provides for the use of an antibody or binding composition which specifically binds to a CCR8, preferably mammalian, e.g., primate, human, cat, dog, rat, or mouse. Antibodies can be raised to various CCR8 proteins, including individual, polymorphic, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms or in their recombinant forms. Additionally, antibodies can be raised to CCR8 proteins in both their native (or active) forms or in their inactive, e.g., denatured, forms. Anti-idiotypic antibodies may also be used.

A number of immunogens may be selected to produce antibodies specifically reactive, or selective for binding, with CCR8 proteins. Recombinant protein is a preferred immunogen for the production of monoclonal or polyclonal antibodies. Naturally occurring protein, from appropriate sources, e.g., primate, rodent, etc., may also be used either in pure or impure form. Synthetic peptides, made

using the CCR8 protein sequences described herein, may also be used as an immunogen for the production of antibodies to CCR8 proteins. Recombinant protein can be expressed and purified in eukaryotic or prokaryotic cells as described, e.g., in Coligan, et al. (eds.) (1995 and periodic supplements) Current Protocols in Protein Science John Wiley & Sons, New York, NY; and Ausubel, et al (eds.) (1987 and periodic supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York, NY. Naturally folded or denatured material can be used, as appropriate, for producing antibodies. Either monoclonal or polyclonal antibodies may be generated, e.g., for subsequent use in immunoassays to measure the protein, or for immunopurification methods.

Methods of producing polyclonal antibodies are well known to those of skill in the art. Typically, an immunogen, preferably a purified protein, is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the CCR8 protein or peptide of interest. For example, when appropriately high titers of antibody to the immunogen are obtained, usually after repeated immunizations, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein can be performed, if desired. See, e.g., Harlow and Lane Antibodies, A Laboratory Manual; or Coligan (ed.) Current Protocols in Immunology. Immunization can also be performed through other methods, e.g., DNA vector immunization. See, e.g., Wang, et al. (1997) Virology 228:278-284.

Monoclonal antibodies may be obtained by various techniques familiar to those skilled in the art. Typically, spleen cells from an animal immunized with a desired antigen are immortalized, commonly by fusion with a myeloma cell. See, Kohler and Milstein (1976) Eur. J. Immunol. 6:511-519. Alternative methods of immortalization include

transformation with Epstein Barr Virus, oncogenes, or retroviruses, or other methods known in the art. See, e.g., Doyle, et al. (eds. 1994 and periodic supplements) Cell and Tissue Culture: Laboratory Procedures, John Wiley and Sons, New York, NY. Colonies arising from single immortalized cells are screened for production of antibodies of the desired specificity and affinity for the antigen, and yield of the monoclonal antibodies produced by such cells may be enhanced by various techniques, including injection into the peritoneal cavity of a vertebrate host. Alternatively, one may isolate DNA sequences which encode a monoclonal antibody or a binding fragment thereof by screening a DNA library from human B cells according, e.g., to the general protocol outlined by Huse, et al. (1989) Science 246:1275-1281.

Antibodies or binding compositions, including binding fragments and single chain versions, against predetermined fragments of CCR8 proteins can be raised by immunization of animals with conjugates of the fragments with carrier proteins as described above. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective CCR8 protein, or screened for Th2 cell depleting ability. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 µM, typically at least about 10 µM, more typically at least about 30 µM, preferably at least about 10 µM, and more preferably at least about 3 µM or better.

In some instances, it is desirable to prepare monoclonal antibodies (mAbs) from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.) Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.)

Academic Press, New York, NY; and particularly in Kohler and Milstein (1975) Nature 256:495-497, which discusses one method of generating monoclonal antibodies. Summarized briefly, this method involves injecting an animal with an immunogen. The animal is then sacrificed and cells taken from its spleen, which are then fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve selection of libraries of antibodies in phage or similar vectors. See, e.g., Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989) Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see, Cabilly, U.S. Patent No. 4,816,567; and Queen, et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033; or made in transgenic mice, see Mendez, et al. (1997) Nature Genetics 15:146-156.

Antibody binding compounds, including binding fragments, of this invention can have significant diagnostic or therapeutic value. They can be useful as non-neutralizing binding compounds and can be coupled to toxins or radionuclides so that when the binding compound binds to the antigen, a cell expressing it, e.g., on its surface, is killed. Further, these binding compounds can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker, and may effect drug targeting.

B. Other Molecules

Antibodies are merely one form of specific binding compositions. Other binding compositions, which will often have similar uses, include molecules that bind with specificity to CCR8 receptor, e.g., in a binding partner-binding partner fashion, an antibody-antigen interaction, or in a natural physiologically relevant protein-protein interaction, either covalent or non-covalent, e.g., proteins which specifically associate with CCR8 receptor protein. The molecule may be a polymer, or chemical reagent. A functional analog may be a protein with structural modifications, or may be a structurally unrelated molecule, e.g., which has a molecular shape which interacts with the appropriate binding determinants.

Drug screening using antibodies or CCR8 or fragments thereof can be performed to identify compounds having binding affinity to CCR8, or can block the natural interaction with ligand. Subsequent biological assays can then be utilized to determine if the compound has intrinsic blocking activity and is therefore an antagonist. Likewise, a compound having intrinsic stimulating activity can signal to the cells via the CCR8 and is thus an agonist in that it simulates the activity of a ligand.

As indicated above, natural ligands for the CCR8 chemokine receptor have been identified. In humans, the I-309 protein (Roos, et al. (1997) J. Biol. Chem. 272:17251-17254; and Tiffany, et al. (1997) J. Exp. Med. 186:165-170;

GenBank M57502 and M57506), the TARC (see Imai, et al. (1997) J. Biol. Chem. 272:15036-15042; GenBank D43767), and in mouse, the TCA-3 (GenBank M17957 and X52401). Mutein antagonists may be developed which maintain receptor binding but lack signaling.

Structural studies of the ligands will lead to design of new variants, particularly analogs exhibiting agonist or antagonist properties on the receptor. This can be combined with previously described screening methods to isolate muteins exhibiting desired spectra of activities.

As receptor specific binding molecules are provided, also included are small molecules identified by screening procedures. Various ligands for the receptor have been identified. In particular, it is well known in the art how to screen for small molecules which interfere, e.g., with ligand binding to the receptor, often by specific binding to the receptor and blocking of binding by natural ligand. See, e.g., meetings on High Throughput Screening, International Business Communications, Southborough, MA 01772-1749. Such molecules may compete with natural ligands, and selectively bind to the CCR8. Such specific binding compounds may be labeled or conjugated to toxic reagents, which are targeted to CCR8+ cells.

These specific binding reagents may similarly be used to target Th2 cells.

III. Immunoassays

Immunoassays are valuable in diagnosing a disease or disorder associated with Th2 imbalance or pathology. Qualitative or quantitative measurement of a particular protein can be performed by a variety of immunoassay methods. For a review of immunological and immunoassay procedures in general, see Stites and Terr (eds.) (1991) Basic and Clinical Immunology (7th ed.). Moreover, the immunoassays of the present invention can be performed in many configurations, which are reviewed extensively in Maggio (ed. 1980) Enzyme Immunoassay CRC Press, Boca Raton,

Florida; Tijan (1985) "Practice and Theory of Enzyme Immunoassays," Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers B.V., Amsterdam; and Harlow and Lane Antibodies, A Laboratory Manual, supra. See also Chan (ed. 1987) Immunoassay: A Practical Guide Academic Press, Orlando, FL; Price and Newman (eds. 1991) Principles and Practice of Immunoassays Stockton Press, NY; and Ngo (ed. 1988) Non-isotopic Immunoassays Plenum Press, NY.

Immunoassays for measurement of CCR8 proteins or peptides can be performed by a variety of methods known to those skilled in the art. In brief, immunoassays to measure the protein can be either competitive or noncompetitive binding assays. In competitive binding assays, the sample to be analyzed competes with a labeled analyte for specific binding sites on a capture agent bound to a solid surface. Preferably the capture agent is an antibody specifically reactive with CCR8 proteins produced as described above. The concentration of labeled analyte bound to the capture agent is inversely proportional to the amount of free analyte present in the sample.

In a competitive binding immunoassay, the CCR8 protein present in the sample competes with labeled protein for binding to a specific binding agent, for example, an antibody specifically reactive with the CCR8 protein. The binding agent may be bound to a solid surface to effect separation of bound labeled protein from the unbound labeled protein. Alternately, the competitive binding assay may be conducted in liquid phase and a variety of techniques known in the art may be used to separate the bound labeled protein from the unbound labeled protein. Following separation, the amount of bound labeled protein is determined. The amount of protein present in the sample is inversely proportional to the amount of labeled protein binding.

Alternatively, a homogeneous immunoassay may be performed in which a separation step is not needed. In these immunoassays, the label on the protein is altered by

the binding of the protein to its specific binding agent. This alteration in the labeled protein results in a decrease or increase in the signal emitted by label, so that measurement of the label at the end of the immunoassay allows for detection or quantitation of the protein.

CCR8 proteins may also be determined by a variety of noncompetitive immunoassay methods. For example, a two-site, solid phase sandwich immunoassay may be used. In this type of assay, a binding agent for the protein, for example an antibody, is attached to a solid support. A second protein binding agent, which may also be an antibody, and which binds the protein at a different site, is labeled. After binding at both sites on the protein has occurred, the unbound labeled binding agent is removed and the amount of labeled binding agent bound to the solid phase is measured. The amount of labeled binding agent bound is directly proportional to the amount of protein in the sample.

Western blot analysis can be used to determine the presence of CCR8 proteins in a sample. Electrophoresis is carried out, for example, on a tissue sample suspected of containing the protein. Following electrophoresis to separate the proteins, and transfer of the proteins to a suitable solid support, e.g., a nitrocellulose filter, the solid support is incubated with an antibody reactive with the protein. This antibody may be labeled, or alternatively may be detected by subsequent incubation with a second labeled antibody that binds the primary antibody.

The immunoassay formats described above may employ labeled assay components. The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. A wide variety of labels and methods may be used. Traditionally, a radioactive label incorporating ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P was used. Non-radioactive labels include ligands which bind to labeled antibodies, fluorophores, chemiluminescent agents, enzymes, and antibodies which can serve as specific binding pair members for a labeled ligand. The choice of

label depends on sensitivity required, ease of conjugation with the compound, stability requirements, and available instrumentation. For a review of various labeling or signal producing systems which may be used, see U.S. Patent No. 4,391,904.

Antibodies reactive with a particular protein can also be measured by a variety of immunoassay methods. Thus modifications of the above procedures may be used to determine the amounts or affinities of various CCR8 antibodies or antibody preparation. For a review of immunological and immunoassay procedures applicable to the measurement of antibodies by immunoassay techniques, see Stites and Terr (eds.) Basic and Clinical Immunology (7th ed.) supra; Maggio (ed.) Enzyme Immunoassay, supra; and Harlow and Lane Antibodies, A Laboratory Manual, supra.

Screens to evaluate the binding and activity of mAbs and binding compositions encompass a variety of methods. Binding can be assayed by detectably labeling the antibody or binding composition as described above. Cells expressing a CCR8 receptor are incubated with this antibody or binding composition, and binding is assayed by Fluorescence Activated Cell Sorting (FACS) analysis.

To evaluate Th2 depletion ability, experimental animals, e.g., mice, are preferably induced to produce those cell types, e.g., by infection with a parasite. Th2 cell counts are made prior to and at various time points after administration of a bolus of the candidate depleting mAb or binding composition. Levels are analyzed in various samples, e.g., blood, serum, nasal or pulmonary lavages, or tissue biopsy staining. A successful depleting mAb or binding composition will significantly lower the level of circulating Th2 cells. Thus, a substantially pure or depleted population will be at least about 85% pure, more preferably at least about 90% pure, and even more preferably at least about 95, 97, or 99% pure.

Evaluation of antibodies can be performed in other animals, e.g., humans using various methods. For example,

blood samples are withdrawn from patients suffering from a Th2 related disease or disorder before and after treatment with a candidate mAb. The antibodies can be used in a diagnostic context to evaluate the extent of Th1 or Th2 polarization, e.g., by FACS, tissue staining, in vitro culture.

IV. Uses

The present invention is useful in the treatment of medical conditions or diseases associated with a Th1 or Th2 cell imbalance. See, e.g., Frank, et al. (eds.1995) Samter's Immunologic Diseases, 5th Ed., vols. I-II, Little, Brown and Co., Boston, MA; Coffman, et al (1989) Science 245:308-310; and Frick, et al. (1988) J. Allergy Clin. Immunol. 82:199-225; each of which is incorporated herein by reference. The binding specificities of the compositions described herein can be administered alone or in combination with another modulator of Th balance, including, e.g., IFN-γ, IL-2, IL-4, antagonists, or other compounds used for the treatment of symptoms, e.g., steroids such as glucocorticoids.

In particular, the selectivity of the CCR8 receptor on these cell types of the CD4+ lineage suggests means to block the functions of the respective cell types. The expression seems to be on the CD4+CD8+ subset (double positive or DP). The expression in this subset may correspond to cells that have already undergone positive selections and are destined, e.g., committed, to become CD4+ thymocytes. As I-309 has been reported to prevent apoptosis in a thymoma (Van Snick, et al. (1996) J. Immunol. 157:2570-2576), the CCR8 may be involved in positive selection in the double positive subset in T cell development. Ligands which agonize or antagonize this may have importance in the regulation of apoptosis in T cell development, as well as Th2 or Th1 dominant response shifting. CCR8 agonists may be used to activate, initiate, or strengthen Th2 mediated responses. Alternatively, antagonists may block the recruitment or attraction of CCR8+

cells to the sites of ligand production, e.g., to the lung or other sites of allergic or asthmatic effects, or to block activation or maintenance of Th2 signaling through the CCR8 receptor.

In a like manner, CD8+ cytotoxic T cells may also have a similar developmental pathway. See O'Garra and Murphy (1994) Curr. Opinion in Immunol. 6:458-466; Palliard, et al. (1988) J. Immunol. 141:849-855; Erard, et al. (1993) Science 260:1802-1805. Whether those cells may signal though the same ligand and receptor pathway remains to be determined.

For example, the ligands for the CCR8 would thus be expected to signal specifically to the cell types expressing the receptor. Thus, it will be possible to block signaling to the Th2 subsets by reagents which block receptor signaling, e.g., antibodies to receptor, antibodies to ligand, and small drug antagonists. The knowledge of the CCR8 mediation of Th2 trafficking provides means to block the attraction by the CCR8 ligands.

Particular medical conditions which are Th1 or Th2 mediated, and are subject to treatment with this invention include, e.g., asthma, allergies, allergic bronchopulmonary aspergillosis, arthritis, inflammatory diseases, IBD, atopic dermatitis, viral infections, various helminthic and parasitic infections, and related conditions. See, e.g., Frank, et al. (eds.1995) Samter's Immunologic Diseases, 5th Ed., vols. I-II, Little, Brown and Co., Boston, MA; Coffman, et al (1989) Science 245:308-310; and Frick, et al. (1988) J. Allergy Clin. Immunol. 82:199-225.

Conversely, it is an unexpected prediction that it will be possible to use known ligands to selectively affect specific functions mediated by Th2 cells. Thus, positive effects, e.g., blocking of apoptotic signals to these cell types become feasible. One known ligand to the CCR8, I309, is expressed in the thymus and there exists some evidence that the ligand can signal an anti-apoptotic signal. Thus, antagonists of the ligand may block the protection,

resulting in cell death. Conversely, blocking of attractive signals to CCR8+ cells may be effected.

Standard immunological techniques are described, e.g., in Hertzenberg, et al. (eds. 1996) Weir's Handbook of Experimental Immunology vols 1-4, Blackwell Science; Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology volumes. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163. These will allow use of the reagents for purifying cell subpopulations, etc.

To prepare pharmaceutical or sterile compositions including the CCR8 specific binding composition, the antibody or binding composition is admixed with a pharmaceutically acceptable carrier or excipient which is preferably inert. Preparation of such pharmaceutical compositions is known in the art, see, e.g., Remington's Pharmaceutical Sciences and U.S. Pharmacopeia: National Formulary, Mack Publishing Company, Easton, PA (1984).

Antibodies or binding compositions are normally administered parenterally, preferably intravenously. Since such protein or peptide antagonists may be immunogenic they are preferably administered slowly, either by a conventional IV administration set or from a subcutaneous depot, e.g. as taught by Tomasi et al, U.S. patent 4,732,863.

When administered parenterally the antibodies or fragments will be formulated in a unit dosage injectable form (solution, suspension, emulsion) in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic and nontherapeutic. The antagonist may be administered in aqueous vehicles such as water, saline or buffered vehicles with or without various additives and/or diluting agents. Alternatively, a suspension, such as a zinc suspension, can be prepared to include the peptide. Such a suspension can be useful for subcutaneous (SQ) or intramuscular (IM) injection. The proportion of antagonist and additive can be varied over a broad range so long as both are present in effective amounts. The antibody is preferably formulated in purified

form substantially free of aggregates, other proteins, endotoxins, and the like, at concentrations of about 5 to 30 mg/ml, preferably 10 to 20 mg/ml. Preferably, the endotoxin levels are less than 2.5 EU/ml. See, e.g., Avis, et al. (eds.)(1993) Pharmaceutical Dosage Forms: Parenteral Medications 2d ed., Dekker, NY; Lieberman, et al. (eds.)(1990) Pharmaceutical Dosage Forms: Tablets 2d ed., Dekker, NY; Lieberman, et al. (eds.)(1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY; Fodor, et al. (1991) Science 251:767-773, Coligan (ed.) Current Protocols in Immunology; Hood, et al. Immunology Benjamin/Cummings; Paul (ed.) Fundamental Immunology; Academic Press; Parce, et al. (1989) Science 246:243-247; Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011; and Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York.

Selecting an administration regimen for an antagonist depends on several factors, including the serum or tissue turnover rate of the antagonist, the level of Th2 depletion, the immunogenicity of the antagonist, the accessibility of the target Th2 cells (e.g., if non-serum Th2 cells are to be blocked). Preferably, an administration regimen maximizes the amount of antagonist delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of antagonist delivered depends in part on the particular antagonist and the severity of the condition being treated. Guidance in selecting appropriate doses is found in the literature on therapeutic uses of antibodies, e.g. Bach et al., chapter 22, in Ferrone et al., (eds.) (1985), Handbook of Monoclonal Antibodies Noges Publications, Park Ridge, NJ; and Russell, pgs. 303-357, and Smith et al., pgs. 365-389, in Haber, et al. (eds.) (1977) Antibodies in Human Diagnosis and Therapy, Raven Press, New York, NY.

Determination of the appropriate dose is made by the clinician, e.g., using parameters or factors known in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than

the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Circulating activated Th2 levels would be important indicators of when an effective dose is reached. Preferably, a CCR8 antibody or binding composition thereof that will be used is derived from the same species as the animal targeted for treatment, thereby minimizing a humoral response to the reagent.

The total weekly dose ranges for antibodies or fragments thereof, which specifically bind to CCR8, range generally from about 1 ng, more generally from about 10 ng, typically from about 100 ng; more typically from about 1 µg, more typically from about 10 µg, preferably from about 100 µg, and more preferably from about 1 mg per kilogram body weight. Although higher amounts may be more efficacious, the lower doses typically will have fewer adverse effects. Generally the range will be less than 100 mg, preferably less than about 50 mg, and more preferably less than about 25 mg per kilogram body weight.

The weekly dose ranges for antagonists, e.g., antibody, binding fragments, range from about 10 µg, preferably at least about 50 µg, and more preferably at least about 100 µg per kilogram of body weight. Generally, the range will be less than about 1000 µg, preferably less than about 500 µg, and more preferably less than about 100 µg per kilogram of body weight. Dosages are on a schedule which effects the desired treatment and can be periodic over shorter or longer term. In general, ranges will be from at least about 10 µg to about 50 mg, preferably about 100 µg to about 10 mg per kilogram body weight.

Other antagonists of the ligands, e.g., muteins, are also contemplated. Hourly dose ranges for muteins range from at least about 10 µg, generally at least about 50 µg, typically at least about 100 µg, and preferably at least 500 µg per hour. Generally the dosage will be less than about 100 mg, typically less than about 30 mg, preferably less than about 10 mg, and more preferably less than about 6 mg

per hour. General ranges will be from at least about 1 µg to about 1000 µg, preferably about 10 µg to about 500 µg per hour.

The present invention also provides for administration of CCR8 antibodies or binding compositions in combination with known therapies, e.g., steroids, particularly glucocorticoids, which alleviate the symptoms associated with excessive Th1 or Th2 responses. Daily dosages for glucocorticoids will range from at least about 1 mg, generally at least about 2 mg, and preferably at least about 5 mg per day. Generally, the dosage will be less than about 100 mg, typically less than about 50 mg, preferably less than about 20 mg, and more preferably at least about 10 mg per day. In general, the ranges will be from at least about 1 mg to about 100 mg, preferably from about 2 mg to 50 mg per day.

The phrase "effective amount" means an amount sufficient to ameliorate a symptom or sign of the Th1 or Th2 condition. Typical mammalian hosts will include mice, rats, cats, dogs, and primates, including humans. An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method, route, and dose of administration and the severity of side affects. When in combination, an effective amount is in ratio to a combination of components and the effect is not limited to individual components alone.

An effective amount of antagonist will decrease the symptoms typically by at least about 10%; usually by at least about 20%; preferably at least about 30%; or more preferably at least about 50%. The present invention provides reagents which will find use in therapeutic applications as described elsewhere herein, e.g., in the general description for treating disorders associated with Th1/Th2 imbalances. See, e.g., Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; Thorn, et al. Harrison's Principles of Internal Medicine,

McGraw-Hill, NY; Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn; Langer (1990) Science 249:1527-1533; and Merck Index, Merck & Co., Rahway, New Jersey.

Antibodies to CCR8 proteins may be used for the identification or sorting of cell populations expressing CCR8 protein, e.g., activated T helper cells. Methods to sort such populations are well known in the art, see, e.g., Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY. Populations of cells expressing the CCR8 receptor can also be purified using magnetic beads as described, e.g., in Bieva, et al. (1989) Exp. Hematol. 17:914-920; Hernebtub, et al. (1990) Bioconj. Chem. 1:411-418; Vaccaro (1990) Am. Biotechnol. Lab. 3:30.

Moreover, antisense nucleic acids may be used. For example, antisense against the ligands may function in a manner like ligand antagonists, and antisense against the CCR8 receptor may function like receptor antagonists. Thus, it may be possible to block the signaling through the pathway with antisense nucleic acids. Conversely, nucleic acids for the receptor may serve as agonists, increasing the numbers of receptor on the cell, thereby increasing cell sensitivity to ligand, and perhaps blocking the normal apoptotic signal described.

Using the assay methods described above, the antibodies or binding compositions are useful in diagnosing diseases states which result in Th1 or Th2 imbalances. Labeled antibodies can also be utilized in analyzing Th2 infiltration in tissues. Antibodies raised against each CCR8 protein will also be useful to raise anti-idiotypic antibodies. These will be useful in d tecting or diagnosing various immunological conditions related to expression of

the respective antigens. Combinations of these signals may be also pursued.

The broad scope of this invention is best understood with reference to the following examples, which are not intended to limit the inventions to the specific embodiments.

EXAMPLES

I. General Methods

Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual, (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; Innis, et al. (eds.)(1990) PCR Protocols: A Guide to Methods and Applications Academic Press, N.Y. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology, vol. 182, and other volumes in this series; manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA; and Coligan, et al. (eds.) (1995 and periodic supplements) Current Protocols in Protein Science, John Wiley & Sons, New York, NY. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to a FLAG sequence or an equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.)

Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QIAGEN, Inc., Chatsworth, CA.

Standard immunological techniques are described, e.g., in Hertzenberg, et al. (eds. 1996) Weir's Handbook of Experimental Immunology vols 1-4, Blackwell Science; Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY; and Methods in Enzymology volumes. 70, 73, 74, 84, 92, 93, 108, 116, 121, 132, 150, 162, and 163. Methodology of cell biology techniques are described, e.g., in Celis (ed. 1998) Cell Biology: A Laboratory Handbook Academic Press, San Diego; and Doyle, et al. (eds. 1994 and periodic supplements) Cell and Tissue Culture: Laboratory Procedures, John Wiley and Sons, New York, NY. Techniques in developmental systems are described, e.g., in Meisami (ed.) Handbook of Human Growth and Developmental Biology CRC Press; and Chrispeels (ed.) Molecular Techniques and Approaches in Developmental Biology Interscience.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

II. Isolation of mammalian CCR8 encoding sequences

The human CCR8 sequence is readily available. See, e.g., Roos, et al. (1997) J. Biol. Chem. 272:17251-17254; and Tiffany, et al. (1997) J. Exp. Med. 186:165-170; GenBank Accession Numbers AF005210 and U45983. See also WO 96/39434.

To isolate the mouse sequence, a 1057 bp fragment containing the entire ORF of the human CCR8 gene was used as probe to screen the murine 129/SV genomic library in the 1/fix vector (Stratagene, La Jolla, CA). 350,000 phage clones were plated. Hybridization with the labeled human CCR8 cDNA was carried out at 37° C in 50% formamide, 5X

SSPE, 10X Denhardt's, 0.1% SDS, and 100 mg/ml salmon sperm DNA for 16 hrs. Filters were washed three times at 50° C in 2X SSC, 0.1% SDS, 20 min. each. Positive genomic phage clones were isolated according to the plaque purification procedure. DNA was extracted, digested by restriction endonucleases, and Southern blot analysis was performed. Hybridizing restriction fragments were subcloned in the pGem11 plasmid (Promega) and sequenced using Sequenase 2.0 (USB). DNA sequence analysis was performed using the DNAsis/Prosis software (Hitachi).

III. Production of cell lines expressing CCR8

Mammalian cells, e.g., NIH3T3, are transfected by electroporation or lipofectamine (Gibco BRL, Gaithersburg, MD) and selected in neomycin supplemented media for two weeks. Resistant colonies are sorted by FACS into 96 well plates and allowed to proliferate. RNA is isolated from individual clones using RNAzol (Friendswood, TX) and analyzed using RT PCR, see, e.g., Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene/Wiley, New York; Innis, et al. (eds.)(1990) PCR Protocols: A Guide to Methods and Applications Academic Press, N.Y., following treatment with DNase.

Positive clones are subject to further analysis in a Ca^{++} flux assay as described, e.g., in Kelner, et al. (1994) Science 266:1395-1399, using I309 or TCA3 as ligand for CCR8. The clone exhibiting the highest Ca^{++} flux is expanded for use in generating mAbs.

Other methods of evaluation of expression can also be utilized, e.g., staining and FACS analysis, tissue staining, northern analysis, etc.

Similarly, the ligands can be recombinantly produced, purchased, or fragments synthetically produced. The ligands will be useful in generating further mutein antagonists or antibodies blocking their effector functions.

IV. Antibody Production

Appropriate mammals are immunized with appropriate amounts of CCR8 transfected cells, e.g., intraperitoneally every 2 weeks for 8 weeks. Typically, rodents are used, though other species should accommodate production of selective and specific antibodies. The final immunization is given intravenously (IV) through the tail vein.

Generic polyclonal antibodies may be collected. Alternatively, monoclonal antibodies can be produced. For example, four days after the IV injection, the spleen is removed and fused to SP2/0 and NS1 cells. HAT resistant hybridomas are selected, e.g., using a protocol designed by Stem Cell Technologies (Vancouver, BC). After 10 days of HAT selection, resistant foci are transferred to 96 well plates and expanded for 3 days. Antibody containing supernatants are analyzed, e.g., by FACS for binding to NIH3T3/CCR8 transfectants. Many different CCR8 mAbs are typically produced. Those antibodies may be isolated and modified, e.g., by labeling or other means as is standard in the art. See, e.g., Harlow and Lane (1988) Antibodies: A Laboratory Manual CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.) Academic Press, New York, NY. Methods to conjugate magnetic reagents, toxic entities, labels, attach the antibodies to solid substrates, to sterile filter, etc., are known in the art.

Additionally, some of the mAbs are used to sort CCR8 positive cells in spleens from mice or primates. Various treatments of the mice are evaluated to determine the relative proportions of Th1 and Th2 cells.

V. Specific expression on Th2 cells

The mAbs may be isotyped, e.g., as directed in an ELISA based kit from Zymed, Inc. (So. San Francisco, CA) and/or in an Ouchterlony based kit from ICN (Aurora, OH). Specific isotypes are selected for the appropriate studies, e.g., for labeling studies or for the complement killing effector function.

Alternatively, the distribution of CCR8, either the human or mouse counterpart, may also be determined by nucleic acid expression analysis. This will indicate the locations of message expression, which typically reflects protein expression levels.

Fluorescent labeling reagents are prepared according to standard methods, e.g., labeled with a fluorescent probe. See, e.g., Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY.

Analysis of human samples can be evaluated in a similar manner. A biological sample, e.g., blood, tissue biopsy sample, lung or nasal lavage, skin punch, is obtained from an individual suffering from a disease or disorder associated with a Th2 mediated disorder. Th2 cell subtype diagnosis is performed, e.g., by FACS analysis, or similar means. A mAb or binding composition, e.g., ligand, which specifically binds to the human CCR8 receptor, is used to label CCR8+ cells.

Mouse counterpart methods may be developed. Highly Th1 or Th2 polarized cell populations may be made as described, e.g., by Openshaw, et al. (1995) J. Exp. Med. 182:1357-1367. Specificity of labeling with the CCR8 reagent can be established.

In human the only significant expression observed was in thymus. There is a slight signal in human spleen, and barely anything in lymph nodes. Mouse data is extrapolated to suggest that there is only significant levels of expression in some T cells; and that the T cell subsets that have been identified as having very high expression of CCR8 in mouse are activated polarized Th2 cells, with some signal in activated NK1.1+ T cells. The latter subset has not been characterized in humans, but we know it is extremely rare in mice, probably <1% of T cells in humans. Thus, the main

significant expression of CCR8 in human T cells, as in mouse, would be Th2-polarized cells.

In addition, the CCR8 goes up very quickly following beginning of Th polarization. So it probably participates in the differentiation and/or commitment of Th2 precurser cells as well as in their recruitment to sites of Th2 accumulation. Also, Th2 cells produce TCA3, the ligand of CCR8 in mouse, suggesting that there is an autologous loop of recruitment of Th2 T cells. Thus, an activated, polarized Th2 T cell will probably be able to recruit other similar cells through its production of TCA-3 (mouse) or I-309 (equivalent ligand in humans).

This polarization process may involve many other factors, and may be assisted or prevented by various other cytokine or chemokine factors. The cytokines involved are well recognized and described above, while the chemokine components include IP-10, MIP-1α, MIP-1β, JE, RANTES, and eotaxin. See Pearlman, et al. (1997) J. Immunol. 158:827-833.

The highly specific labeling of Th2 cells will be useful in various contexts. It provides a means for positive selection of CCR8 bearing cells, being of the T cell Th2 subtype. The specificity of expression on Th2 cells also allows for the targeting of CCR8 specific reagents. Thus, antibodies which bind to CCR8 will bring them specifically to the Th2 subset. Thus, toxic conjugates may be used to selectively or preferentially kill the Th2 subset. Alternatively to antibodies, a CCR8 specific ligand, e.g., the I-309 or TCA3 ligands may be conjugated to a toxic compound.

Blocking of the ligand-CCR8 interaction or signaling will likely have specific effects on Th2 cells, e.g., chemoattraction, development, or physiology of the subtype. Thus, agonists or antagonists of the I-309 or TCA3 ligands should have specific effects on Th2 cells.

VI. Selecting Th2 cells

CCR8+ cells may be isolated. Standard methods exist to isolate T cells, and other cells expressing specific surface markers. FACS and magnetic bead methods will be applicable. See, e.g., Hertzenberg, et al. (eds. 1996) Weir's Handbook of Experimental Immunology vols 1-4, Blackwell Science; and Coligan (1991) Current Protocols in Immunology Wiley/Greene, NY.

The isolated Th2 cells may be proliferated ex vivo, where appropriate, e.g., for reintroduction back to the same or another patient. The Th2 cells may be transfected with a desired gene or vector.

The CCR8+ specificity may be taken advantage of, e.g., to specifically deplete the Th2 cells. Conjugation of a CCR8 specific binding agent to a toxic moiety may allow for selective destruction of Th2 cells, where appropriate. Antagonists may block chemoattraction of Th2 cells to the sites of ligands for the receptor.

VII. CCR8 Antagonists

Various antagonists of the CCR8 are available. For example, antibodies against the receptor itself may block the binding of ligand, thereby serving as a direct receptor antagonist. Other antagonists may function by blocking the binding of ligand to receptor, e.g., by binding to the ligand in a way to preclude the possibility of binding to the receptor. Other antagonists, e.g., mutein antagonists, may bind to the receptor without signaling, thereby blocking a true agonist from binding. Many of these may serve to block the signal transmitted to the CCR8 bearing cells, specifically Th2 cells. Thus, means are provided to block physiological signals transduced through CCR8, which is selectively expressed on Th2 cells.

In addition, the CCR8 specific reagents are useful in targeting CCR8+ cells, which are also TH2 cells. Thus, toxic conjugates will selectively deplete CCR8+ cells from a population. This may be useful in treating parasitic

infections, which typically involve a cellular response (Th1 type). The deletion of Th2 cells removes the accompanying suppression of Th1 effector functions.

Information on the criticality of particular residues is determined using standard procedures and analysis. Standard mutagenesis analysis is performed, e.g., by generating many different variants at determined positions, e.g., at the positions identified above, and evaluating biological activities of the variants. This may be performed to the extent of determining positions which modify activity, or to focus on specific positions to determine the residues which can be substituted to either retain, block, or modulate biological activity.

Alternatively, analysis of natural variants can indicate what positions tolerate natural mutations. This may result from populational analysis of variation among individuals, or across strains or species. Samples from selected individuals are analyzed, e.g., by PCR analysis and sequencing. This allows evaluation of population polymorphisms.

All citations herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled; and the invention is not to be limited by the specific embodiments that have been presented herein by way of example.

WHAT IS CLAIMED IS:

1. A substantially pure CCR8+ population of cells which consists essentially of Th2 cells.

2. The population of Claim 1, which produce low amounts of the cytokines IL-2 and IFN-γ; and produce high amounts of IL-4 and IL-10.

3. The population of Claim 1, which bind anti-CCR8 antibody or antiserum.

4. The population of Claim 1, prepared by Fluorescent Activated Cell Sorting with a labeled CCR8 selective:

a) ligand;

b) antibody; or

c) binding compound comprising the antigen binding portion from an antibody which selectively binds CCR8.

5. The population of Claim 4, wherein:

a) said ligand is I-309 or TCA3; or

b) said antibody binds to human CCR8.

6. A method of selectively labeling Th2 cells comprising the step of contacting a population comprising said cells to a reagent which specifically binds to a CCR8.

7. The method of Claim 6, wherein said population comprises Th1 cells.

8. The method of Claim 6, further comprising selectively depleting said Th2 cells from said population.

9. The method of Claim 8, wherein said depleting is:

a) by killing of said cells expressing CCR8;

b) magnetic bead depletion of said Th2 cells; or
c) FACS sorting of said Th2 cells.

10. The method of Claim 9, wherein said reagent is a toxic conjugate with:
a) a ligand specific for CCR8 binding;
b) a binding compound comprising an antigen binding portion from an antibody which selectively binds to a mammalian CCR8; or
c) a binding composition comprising antigen binding portions from antiserum which selectively binds to a mammalian CCR8.

11. The method of Claim 9, wherein said reagent is a monoclonal antibody which selectively binds to human CCR8.

12. The cells prepared by the method of Claim 6.

13. A method of modulating a physiological signal specifically to Th2 cells, comprising contacting said Th2 cells with a CCR8 agonist or antagonist.

14. The method of Claim 13, wherein said modulating is:
a) blocking, and said contacting is with a CCR8 antagonist;
b) inducing, and said contacting is with a CCR8 signaling agonist; or
c) directing a response between a Th1 and Th2 response, and said contacting is with a CCR8 antagonist or CCR8 signaling agonist.

15. The method of Claim 14, wherein said antagonist is an antibody.

16. The method of Claim 15, wherein said antibody binds to a CCR8 ligand or to CCR8.

17. The method of Claim 14, wherein said CCR8 agonist is I-309 or TCA3.

18. The method of Claim 14, wherein said modulating is directing said response to a Th2 response, and said contacting is with said CCR8 signaling agonist.

19. The method of Claim 13, wherein said physiological signal is a proliferation, apoptotic, differentiation, or chamoattraction signal.

20. The method of Claim 13, wherein said contacting is with another chemokine or cytokine agonist or antagonist, including IL-12, IL-12 antagonist, IL-1γ, or IL-1γ antagonist.